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Plastics — Assessment of the effectiveness of fungistatic compounds in plastics formulations

Plastiques — Évaluation de l'efficacité des composés fongistatiques dans les formulations de plastiques

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16869 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 6, *Ageing, chemical and environmental resistance*.

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This second edition cancels and replaces the first edition (ISO 16869:2001), of which it constitutes a minor technical revision. The main changes are an increase in the maximum diameter of the test specimen to 4 cm (see 6.1) and the introduction of centrifuging operations in the preparation of the spore suspension in 8.4.1.

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Introduction

It is a well known phenomenon that plasticizers as well as other ingredients in plastics formulations can be attacked by bacteria, yeasts and fungi, the latter being the most important deteriogens. Microbial attack results in a reduction of the quality of the plastic, causing embrittlement as well as discoloration. This deterioration is of economic importance.

The prevention of fungal attack can be achieved by the incorporation of a fungistatic compound into the formulation. The function of this fungistat is to inhibit the growth of any fungi present on the surface of the plastic product.

The method described in this International Standard determines the effectiveness of fungistatic compounds incorporated into the plastic against the fungi used in the test.

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WARNING — Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence and may be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests. Codes of practice for disinfection, sterilization and personal hygiene must be strictly observed.

It is recommended that workers consult IEC 60068-2-10:2005, Annex A "Danger to personnel", and ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations.*

1 Scope

This International Standard specifies a method for determining the effectiveness of fungistatic compounds in protecting susceptible ingredients like plasticizers, stabilizers, etc., in plastics formulations. The method demonstrates whether or not a plastic product is actively protected against fungal attack.

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The evaluation is by visual examination.

The test is applicable to any articles made of plastic that are in the form of films or plates no thicker than 10 mm. In addition, porous materials such as plastic foams may be tested provided that they are in the above-mentioned form.

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A minimum diffusion of the fungicides that migrate out of the matrix is necessary with this procedure.

In contrast to ISO 846, the test films are not sprayed with a fungal spore suspension but covered with a layer of test agar containing spores. It has been found that this leads to a better distribution of the spores as well as providing a good supply of water necessary for spore germination on plastic surfaces that are normally hydrophobic.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 291:2008, *Plastics — Standard atmospheres for conditioning and testing*

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3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
plastic susceptible to fungal attack
plastic material that contains in its formulation one or more nutrients that support fungal growth

3.2
fungistat
compound that prevents fungal growth on a material that is normally susceptible to fungal attack

4 Principle

Test specimens are exposed to a suspension of mixed fungal spores. The spores are applied to the surface of the test specimen in a thin layer of an agar medium without an added carbon source. In this way, uniform distribution of the spores is achieved as well as an optimum supply of water.

The absence of fungistatic agents in the plastic material will lead to germination of the fungal spores and initial growth. When the ingredients in the material are susceptible to fungal attack and no active fungistat is included in the formulation, further growth and sporulation will occur over and around the test specimen.

The presence of an active fungistat in the material will lead to suppression of spore germination and initial growth in the area over and around the test specimen. Fungistatic agents can migrate into the agar around the test specimen, thereby suppressing germination and appearing to give an increased zone of inhibition.

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Although not relevant to the interpretation of the test results, the inhibition zone can be an indication of the behaviour of the fungistat under test.

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5 Apparatus and materials

5.1 Apparatus

Sterilize all glassware and all parts of the rest of the apparatus that will come into contact with the culture media and/or reagents (except those which are supplied sterile) by one of the following methods:

method A: autoclave (see 5.1.2) at 121 °C for a minimum of 15 min;

method B: use a dry-heat sterilizer (see 5.1.2) at 180 °C for at least 30 min, at 170 °C for at least 1 h, or at 160 °C for at least 2 h;

method C: use a membrane-filtration system of pore size 0,45 µm.

5.1.1 Incubator, maintained at 24 °C ± 1 °C.

5.1.2 Sterilization apparatus:

5.1.2.1 For moist-heat sterilization, a suitable **autoclave**.

5.1.2.2 For dry-heat sterilization, a **hot-air oven** maintained at one of the temperatures specified in method B above.

5.1.2.3 For membrane-filtration sterilization, a **membrane-filtration apparatus**, of pore size as specified in method C above.

5.1.3 Analytical balance, accurate to ± 0,1 mg.

5.1.4 Laboratory centrifuge, speed 2 000 rpm to 5 000 rpm.

5.1.5 Counting chamber (for direct counting with the help of a microscope).

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5.1.6 Microscope, magnification × 100.

5.1.7 pH-meter, having an accuracy of ± 0,1 pH-units, capable of temperature correction.

5.1.8 Vortex shaker, operating at 2 000 rpm to 2 500 rpm.

5.1.9 Containers: test tubes, flasks or bottles of suitable capacity.

5.1.10 Petri dishes, 90 mm to 100 mm in diameter and at least 15 mm deep.

5.1.11 Graduated pipettes, with nominal capacities of 1,0 ml and 15,0 ml. Calibrated automatic pipettes may be used.

5.1.12 Graduated measuring cylinder, minimum capacity 30 ml.

5.1.13 Glass beads, diameter 3 mm to 5 mm.

5.2 Culture media and reagents

All reagents shall be of analytical grade and/or of a grade appropriate for microbiological purposes.

5.2.1 Water

Any water used shall be distilled or deionized and have a conductivity of < 1 µS/cm.

5.2.2 Malt-extract agar (MEA)

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Malt extract	30,0 g
Soya peptone	3,0 g
Agar-agar	15,0 g
Water (5.2.1)	to make up to 1 000 ml

Sterilize in the autoclave (see 5.1.2). After sterilization, the pH of the medium shall be 7,0 ± 0,2.

5.2.3 Chaetomium agar

$NaNO_3$	2,0 g
$MgSO_4 \cdot 7H_2O$	0,5 g
KCl	0,5 g
$Fe_2(SO_4)_3 \cdot H_2O$	0,01 g
KH_2PO_4	0,14 g
K_2HPO_4	1,20 g
Agar-agar	15,0 g
Yeast extract	0,02 g
Microcellulose	20,0 g
or	
Carboxymethyl-cellulose (Na salt)	10,0 g
Water (5.2.1)	to make up to 1 000 ml

Sterilize in the autoclave (see 5.1.2). After sterilization, the pH of the medium shall be 7,2 ± 0,2.

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